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FINAL REPORT

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NEUROMUSCULAR DEVELOPMENT AND REGULATION OF MYOSIN EXPRESSION

Sue Bodine, Ph.D.
Regeneron Pharmaceuticals
777 Old Saw Mill River Rd.
Tarrytown, New York 10591-6707

914-345-7755

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The proposed experiments were designed to determine whether the absence of gravity during embryogenesis influences the postnatal development of the neuromuscular system. Further, we examined the effects of reduced gravity on hindlimb muscles of the pregnant rats.

HYPOTHESES

Microgravity may have short and long-term effects on the development of muscle fiber type differentiation and force producing capabilities.

Microgravity will reduce muscle fiber size and cause a shift in myosin heavy chain expression from slow to fast in hindlimb muscles of the adult pregnant rats.

OBJECTIVE

Examine the time course of myosin heavy chain expression and the development of adult fiber type in hindlimb muscles which have been exposed to microgravity during embryonic development (G11-20).

Define the degree of muscle atrophy and fiber type changes that occur in muscle fibers of the soleus, medial gastrocnemius and tibialis anterior of the adult rats following 9 days of microgravity.

EXPERIMENTAL DESIGN

Nulliparous pregnant female rats (Sprague Dawley) were utilized as the experimental animal. Dams were shipped and arrived at Kennedy Space Center on Gestational day 2 (G2). On G10, approximately 19 hours prior to launch, a total of 10 dams were loaded into 2 AEM cages (5 dams/AEM) and transported to the Middeck locker area of the Space Shuttle. Launch occurred on G11. Landing of the Space Shuttle occurred on G20 (9 day flight).

On the day of recovery, 4 dams were anesthetized with isoflurane and underwent cesarean delivery. The remaining dams were allowed to deliver naturally. Fetuses and pups were taken at the following times.

AGE	TISSUE
G20	Right and Left Hindlimbs
PN1	Right and Left Hindlimbs
PN3	Right and Left Hindlimbs
PN7	Right and Left Hindlimbs
PN14	Right and Left Hindlimbs
PN21	Right and Left Sol, MG, EDL, TA
PN35	Right and Left Sol, MG, EDL, TA
R+0, Dams	Right and Left Sol, MG, EDL, TA
R+4, Dams	Right and Left Sol, MG, EDL, TA

DATA ANALYSIS

The analysis of the tissues collected from the fetuses and pups was not completed due to the early termination date of this grant. The fetal and postnatal tissues were transferred to Dr. Kathleen Clark at the University of Michigan for analysis. We examined the embryonic and neonatal myosin heavy chain mRNA expression in the fetal tissues and found no difference in the flight and non-flight animals.

The fiber cross-sectional area and fiber type distribution of the soleus and medial gastrocnemius from the adult pregnant rats were analyzed. A summary of the data follows.

Fiber Cross-Sectional Area: The mean fiber cross-sectional area of each muscle was determined from a population of 100 to 400 fibers measured from a serial cross-section immunohistochemically stained with a monoclonal antibody for laminin which stains the basal lamina just outside the plasma membrane. A Vectastain ABC™ kit (Vector Labs, Burlingame, CA, USA) was used to amplify the antigen-antibody complex, which in turn, was visualized by treatment with a diaminobenzidine (DAB) peroxidase reaction. Using an image analysis system (Image I-AT, Universal Imaging Corporation), the region within the laminin boundaries was identified and the fiber cross-sectional area was calculated in μm^2 .

Fiber Types: Fibers were classified as type I, IIa, IIb, IIx or hybrid (coexpression of slow and fast) using monoclonal antibodies which label the different myosin heavy chain (MHC) isoforms. Serial cross-sections were incubated with primary antibodies (BA-F8, BF-13, BF-35 and SC-71 generously donated by S. Schiaffino (Padova, Italy)) overnight at 25°C. Sections incubated without primary antibody were used as controls to visualize non-specific labeling. A Vectastain ABC™ kit (Vector Labs, Burlingame, CA, USA) was used to amplify the antigen-antibody complex, which in turn, was visualized by treatment with a DAB peroxidase reaction. Serial sections also were stained for hematoxylin and eosin (H&E) for routine histological examination.

RESULTS

Fiber Cross-Sectional Area:

There was no significant decrease in the mean fiber size in the soleus or medial gastrocnemius following a 9-day flight (Table 1). This is contrary to what has been reported in the literature for normal adult rats following exposure to microgravity.

TABLE 1:

GROUP	n	MEAN	SD
MG, Control	4	1692	439
MG, Flight	4	1969	321
Sol, Control	4	1396	81
Sol, Flight	4	1275	191

Fiber Type

Muscle fibers were classified as type I, IIa, IIx or hybrid (coexpression of slow and fast) using monoclonal antibodies which label the different myosin heavy chain (MHC) isoforms.

Muscle fibers could be classified into four types based on their immunohistochemical staining to monoclonal antibodies to the myosin heavy chain. The type I fibers were positive for the BA-P8 (only slow MHC) and BF-35 (all MHCs except IIx) antibodies, and negative for the BF-13 (all type II MHCs) and SC-71 (only IIa MHC) antibodies. The type IIa fibers were positive for all of the antibodies except the F-8 antibody which is specific for the slow MHC. The classification of IIx was based on the negative staining of fibers for the BF-35 antibody. These fibers were also negative for BA-P8, positive for BF-13 and intermediate for SC-71. Hybrid fibers stained positively for all the antibodies and presumably expressed both type I and IIa MHCs.

There was no change in the percentage of fast and slow fibers in the medial gastrocnemius muscle. In the soleus there was a significant increase in the percentage of fibers which expressed both fast and slow fibers (Table 2).

TABLE 2:

FIBER TYPE	SOL, control	SOL, flight
I	84 ± 2	77 ± 4
IIa	10 ± 2	10 ± 3
IIx	6 ± 1	13 ± 1